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ELECTRONIC ASPECTS OF THE MECHANISMS OF THE LETHAL AND MUTAGENIC ACTION OF ULTRAVIOLET LIGHT

G. G. Dyadyusha, V. I. Danilov and O. V. Shramko

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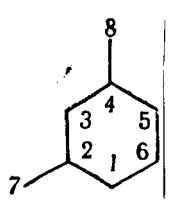
ABSTRACT. The lowest triplet states of pyrimidine bases (PB) and PB derivatives have been studied by the self-consistent-field open-shell and closed-shell methods. Triplet excitation is almost completely localized on the 5-6 bond; this fact favors the reaction of photodimerization. A correlation between the degree of triplet state localization on the 5-6 bond and the ease of photodimerization is demonstrated. Investigation of the ground state of cytosine and cytosine hydrate in neutral and cationic forms shows that the deamination reaction of cytosine hydrate proceeds through the protonic stage. The relationship between the data obtained and the mutagenic effect of ultraviolet light is discussed.

The influence of ultraviolet light on bacteria, bacteriophages, and animal/539* cells causes lethal and mutagenic effects. The basis of the lethal effect of ultraviolet light [1, 2] is the formation of various dimers of pyrimidine bases (PBs) which block the synthesis of nucleic acids, causing loss of reproductive ability. The mutagenic effect of ultraviolet light is related to the deamination of cytidylic acid hydrates formed as a result of irradiation or of PB dimers containing cytosine [3, 4].

The available data made it possible to assume [5] that PB dimerization proceeds through the triplet state; practically nothing is known of the causes of the marked tendency toward deamination of cytosine after radiation. In this connection, there is a particular interest in the study of the electron structure of PBs and PB derivatives in the ground and excited states. Mantione and Pullman [6] made such an attempt. A study of the first excited state of PBs and some PB derivatives established a correlation between the ease of photodimerization and the density of unpaired electrons in atoms 5 and 6 (see diagram for numeration of atoms).



However, the method used in [6] does not permit the triplet and singlet excited states to be distinguished, although understanding of the dimerization

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mechanism requires direct study of the lowest excited states (singlet and triplet), which are π-π^* states [7].

We demonstrated earlier [8, 9] that the PB triplet state meets the conditions necessary for the dimerization reaction; these conditions are more favorable than in the singlet excited state. The results of a quantum-mechanical calculation of the π-electron structure of the lowest triplet state of PBs and some PB derivatives are analyzed in the present work by the self-consistent-field open-shell method [10-12].

Discussion of Results

The table presents the most essential calculated quantities for the problem under consideration. All compounds studied (with the exception of 6-azothymine, which will be discussed on the following page) are listed in order of increasing p_{5-6} and simultaneously decreasing ρ_5 and ρ_6, i.e., by the degree of localization of excitation on the 5-6 bond. /540

According to our calculations (see table), the 5-6 bond is practically double in the ground state of all compounds studied. With transition to the triplet state, p_{5-6} decreases significantly, indicating that in PB and some PB derivatives this π-bond becomes so weak that it can be considered broken. Comparison of q_5 and ρ_5, and also q_6 and ρ_6, shows that the electron density in these atoms is mainly a function of unpaired electron density because of strong concentration of the latter in atoms C_5 and C_6. This considerably increases the reactivity of a number of the molecules studied, since this triplet state is an unsaturated valence state [13]. The lowest π-π^* triplet state, consequently, has the requisites for PB dimerization, which is to say rupture of the 5-6 π-bond, followed by the formation of a cyclobutane ring.

All compounds listed in the table can be divided by degree of localization of excitation on the 5-6 bond into three groups. The first group contains the first four compounds, which are known to be easily dimerized [14]. The second group contains the next three compounds, which are dimerized with less ease [14]. The third group consists of compounds which are not dimerized or are dimerized with great difficulty; we have placed isocytosine in this group. Unfortunately, the literature contains no quantitative data on the study of photodimerization of isocytosine; it is only noted [14] that it dimerizes to a lesser degree than 6-methyluracil or uracil. A more detailed investigation of its dimerization would be of interest. Our results for this compound differ significantly from those obtained in [6]. According to our data, localization of unpaired electron density in atoms 5 and 6 is considerably less than in compounds of the first and second groups, because the density in the O_8 atom (ρ_8 = 0.78) increases. This redistribution of unpaired electron density is not noted in [6], and the authors consider the degree of dimerization of isocytosine to be average. Our results also differ from those of [6] with regard to 2-thiothymine. Although the results of both studies indicate the nondimerization of this compound, there is, nevertheless, considerable difference between them; /541

ELECTRON INDICES OF PYRIMIDINE DERIVATIVES IN THE GROUND AND EXCITED STATES.

Compounds	State	p_{5-6}	ρ_5	ρ_6	q_5	q_6
uracil	S_0	0.900	—	—	1.064	0.942
	T	0.085 (0.332)	0.822 (0.554)	0.875 (0.647)	0.926 (0.831)	1.086 (1.078)
6-methyluracil	S_0	0.870	—	—	1.080	0.927
	T	0.100 (0.321)	0.820 (0.579)	0.802 (0.578)	0.923 (0.841)	1.074 (1.053)
thymine	S_0	0.875	—	—	1.047	0.958
	T	0.106 (0.374)	0.723 (0.486)	0.871 (0.669)	0.928 (0.877)	1.085 (1.085)
orotic acid	S_0	0.859	—	—	1.099	0.869
	T	0.119 (0.378)	0.787 (0.525)	0.811 (0.544)	0.888 (0.781)	1.046 (1.005)
5-aminouracil	S_0	0.861	—	—	1.044	1.037
	T	0.134 (0.379)	0.616 (0.375)	0.864 (0.641)	1.021 (0.910)	1.085 (1.041)
cytosine	S_0	0.846	—	—	1.090	0.901
	T	0.144 (0.524)	0.664 (0.250)	0.842 (0.513)	0.924 (0.990)	1.102 (1.148)
5-methylcytosine	S_0	0.825	—	—	1.072	0.912
	T	0.165 (0.469)	0.561 (0.273)	0.837 (0.559)	0.920 (0.957)	1.101 (1.144)
5-nitrouracil	S_0	0.809	—	—	1.077	0.860
	T	0.265 (0.430)	0.382 (0.357)	0.774 (0.560)	0.828 (0.814)	1.046 (1.020)
2-thiothymine	S_0	0.876	—	—	1.006	0.994
	T	0.314 (0.571)	0.254 (0.277)	0.768 (0.408)	0.908 (0.776)	1.106 (0.951)
isocytosine	S_0	0.890	—	—	1.010	0.979
	T	0.388 (0.687)	0.169 (0.194)	0.722 (0.292)	0.958 (0.841)	1.118 (0.955)
6-azothymine	S_0	0.848	—	—	0.912	0.178
	T	0.107 (0.302)	0.735 (0.512)	0.778 (0.587)	0.933 (0.894)	1.197 (1.262)

Note: S_0 = ground singlet state; T = first triplet state; p_{5-6} = order of 5-6 bond; $\rho_5(\rho_6)$ and $q_5(q_6)$ = density of unpaired electrons and electron density in atom 5 [6].

i.e., the significant decrease of ρ_5 was related in [6] to the concentration of unpaired electron density in the S_7 atom, while in our study, it is attributed to concentration in O_8 ($\rho_8 = 0.65$).

Thus, this review indicates that the ease of PB dimerization is correlated with both the density of unpaired electrons in atoms 5 and 6, and the order of the 5-6 bond.

Returning to 6-azothymine, we see that neither the calculations in [6] nor our own calculations, explain the absence of its photodimerization. It is possible that the introduction into thymine of the nitrogen atom with its unshared pair of n-electrons results in the appearance of new singlet and triplet n-π^* transitions occurring at energy levels lower than the corresponding π-π^* transitions. As a result, entry into the triplet n-π^* state following excitation is most probable; apparently localization of excitation on the 5-6 bond does not occur in this state.

For a comparison with the above described results, the table also contains (in parentheses) the results of calculation by the self-consistent-field closed-shell method. Comparison of the calculation results obtained by the two methods reveals marked differences. The results of calculations based on the closed-shell theory provide contrasting arrangements of PB ranked by ease of dimerization, depending on whether the latter is measured by the distribution of ρ_5 and ρ_6, or by p_{5-6}. If ease of dimerization is based on excitation localization on the 5-6 bond, use of the closed-shell theory causes uracil to change places with 6-methyluracil, and cytosine to change places with 5-methylcytosine. However, the basic difference is that the open-shell theory predicts a large localization of triplet excitation on the 5-6 bond. Besides giving better agreement with the experimental data [7], the open-shell theory provides a more correct physical result for the chosen system of integrals: the energy of the first triplet states decreases by 0.5-1 eV in comparison with energies computed without reminimization of wave functions.

In addition to dimers formed during ultraviolet irradiation of organisms, nucleotide hydrates are important photoproducts [1]. Since the mutagenic effect is related to the formation of cytosine hydrates and their subsequent deamination, it is clear that study of cytosine hydrate is important for an understanding of the causes of the increased tendency to deaminization.

We studied the ground state of cytosine hydrate by the self-consistent-field method. It was found that the C_4 atom, through which deaminization proceeds, has a charge of +0.23 in cytosine hydrate, while cytosine itself has a charge of +0.19, i.e., deaminization during hydrate formation is facilitated to a certain extent. However, calculation of the nucleophilic localization energy /542 of the C_4 atom, which is 12.76 eV in cytosine and 12.95 eV in cytosine hydrate, gives the opposite picture. This seems to render improbable the viewpoint of Mantione and Pullman [6], who explain the increased tendency deaminization by the increase in the positive charge on the C_4 atom due to simple saturation of the 5-6 bond.

Much more important to the facilitation of cytosine deaminization, in our opinion, is the fact that the formation of cytidylic acid hydrate significantly increases the pH of the dissociating group of the base. Thus, according to the data in [3], the pH of cytidylic acid hydrate is 5.56, while the pH of cytidylic acid is 4.26, i.e., if the relative content of cationic and neutral forms in cytidylic acid is 1:1000, it is 1:50 in the hydrate. In this connection, it is of interest to study the ability of the cationic form of cytosine hydrate to deaminize. We computed the electronic structure of the cationic forms of cytosine hydrate and of cytosine itself. The charge on the C_4 atom in the cation of cytosine hydrate increased very sharply (by 0.39), while the energy of nucleophilic localization of this atom decreased substantially (by 3.50 eV) in comparison with the neutral form of cytosine hydrate. Also observed were a noticeable increase of the positive charge and a decrease of the localization energy of the C_4 atom in the protonic form of cytosine hydrate in comparison with the protonic form of cytosine itself (in the latter, the C_4 atom has a charge of +0.46, while the localization energy is 11.09 eV). This greatly favors the deaminization reaction.

Actually, experimental data [3] indicate that the greatest yield of the hydrated form of uridic acid obtained as a result of deaminization of cytidylic acid hydrate is observed at pH 3; i.e., when the latter is almost completely found in the protonic form. As the pH increases, the rate of deaminization decreases, but not as quickly as should be expected from the decrease in concentration of the protonic form; this is due to a simultaneous increase in the concentration of OH^- ions which are the most probable agents of deaminization. On the basis of the data obtained, there is reason to suppose that the process of cytosine deaminization in uracil takes place mainly in the protonic form of cytosine hydrate.

This process is capable of explaining part of the experimental data on the mutagenic effect of ultraviolet light [15, 16], which led to the substitution of a pair of guanine-cytosine bases (GC) for a pair of adenine-thymine bases (AT). It is possible that the above-described deaminization mechanism also occurs in dimers containing cytosine. Some of the GC → AT transitions are evidently controlled by change in the coding properties of cytosine hydrate.

It is possible that in the reduplication of nucleic acids, certain cationic forms of cytosine hydrate do not pair with any of the bases, which may result in deletion. This possibility, together with the occurrence of deletions due to the formation of PB dimers, may explain some of the mutations which were not identified [15, 16], but were assumed to be deletions or insertions.

The mechanisms under consideration do not explain 6% of the mutations attributed to AT → GC transitions [16]. Apropos, it is of interest to investigate the hydrated form of uracil and its matrix properties, since possible decrease in the pH of its dissociative group in the hydrate could lead to the inclusion of guanine, which would explain the occurrence of AT → GC transitions. Following exposure to ultraviolet radiation a shift of the tautomeric equilibrium of A or T in the matrix accompanying replication errors, or of G or C in the substrate accompanying inclusion errors might also have the same effect.

As the computations which one of us performed [17] indicate, the tautomeric equilibrium of T and U in the lowest excited singlet state is displaced in the /543 direction of lactam forms by comparison with the ground state equilibrium.

Conclusions

A quantum-mechanical calculation of the ground and excited states of various pyrimidine derivatives supports the conclusion that triplet excitation is almost completely localized on the 5-6 bond; the latter, with simultaneous rupture of the π-bond, is a necessary condition of cyclobutane ring formation.

The compounds studied show a correlation between the degree of localization of triplet excitation on the 5-6 bond and ease of photodimerization.

The study of cytosine and cytosine hydrate in neutral and cationic forms indicates that the deaminization reaction of cytosine is considerably facilitated in the protonic form of the hydrate. This process is capable of explaining some of the experimental data on the mutagenic effect of ultraviolet light.

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